30. A process according to any of claims 1-29 wherein the protein is capable of having at least 1, such as at least 2, at least 3, at least 4, at least 5, or at least 6 disulphide bonds.

31. A process according to any of claims 1-30 wherein the protein is capable of having at most 20, such as at most 15, at most 10, at most 8, at most 5, at most 4, at most 3, or at most 2 disulphide bonds.

32. A process according to any of claims 3-31 wherein the cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell and a plant cell.

33. A process according to any of claims 1-32 wherein the cell is a bacterial cell selected from the group consisting of a gram positive bacterium and a gram negative bacterium.

34. A process according to claim 33 wherein the gram negative bacterium is *E. coli* including a strain BL21 or a derivative thereof or a strain XA90 or a derivative thereof.

35. A process according to any of claims 1-34 wherein the cell is genetically modified to have a less reducing intracellular environment than a non-modified cell of the same strain.

36. A process according to any of claims 1-35 wherein the cell has been modified to have a reduced or lacking activity of a thioredoxin reductase or an enzyme having a similar effect on the sulfhydryl reducing potential of the cytoplasm.

37. A process according to any of claims 1-36 wherein the modified cell is a *trxB*- mutant.

38. A process according to any of claims 1-37 wherein the gene is a derivative of a naturally occurring gene.

39. A process according to claim 38 wherein the derivative is obtained by substituting at least one codon which is used more frequently by the host cell than the one originally present where the codon codes for the same amino acid.

40. A process according to any of claims 1-39 wherein the gene is under control of a regulatory DNA sequence not naturally associated with the gene.

41. A process according to any of claims 1-40 wherein the bacterial cell is transformed with an expression vector selected from the group consisting of pET vectors, e.g. T7 promoters.

42. A stable peptide free MHC protein obtainable by a process according to any of claims 1-41.

43. A kit comprising a MHC class I heavy chain and a β_2m allowing the recipient to produce and measure or detect a functional MHC class I protein to which a peptide, which is capable of binding to said MHC class I protein, can be added leading to the generation of a functional MHC class I protein.

44. A kit according to claim 43 which comprises labelling of one or more MHC class I subunits (heavy chain, b2m and/or peptide) to measure or detect the generation of the MHC class I protein.

45. A kit according to claim 43 and 44 wherein the measurement or detection system of the generated MHC class I protein is selected from the group of technologies consisting of radio-ligand, immuno-precipitation, ELISA, plasmon resonance, fluorescence polarization, analytical ultracentrifugation, biochemical precipitation, ultrafiltration, chromatography and equilibrium dialysis.

46. A kit according to claims 43-45 which comprises an oligomerization of MHC proteins, such as two, three, four or more.

47. A kit according to claims 43-46 wherein a further reagent is added as a marker making the kit suitable for diagnostic purposes.

48. A kit according to claim 44-47 wherein the marker is selected from the group consisting of biotin, fluorochromes, enzymes, chemiluminescense, and radioactive markers.

49. Use of a process according to any of claims 1-41 in the manufacturing of MHC.

50. Use of a stable empty MHC protein according to claim 42 in analysis of the effect of changing an amino acid in the MHC on the binding specificity of said MHC as assessed by an analysis using a peptide library approach be it a synthetic or recombinant library.

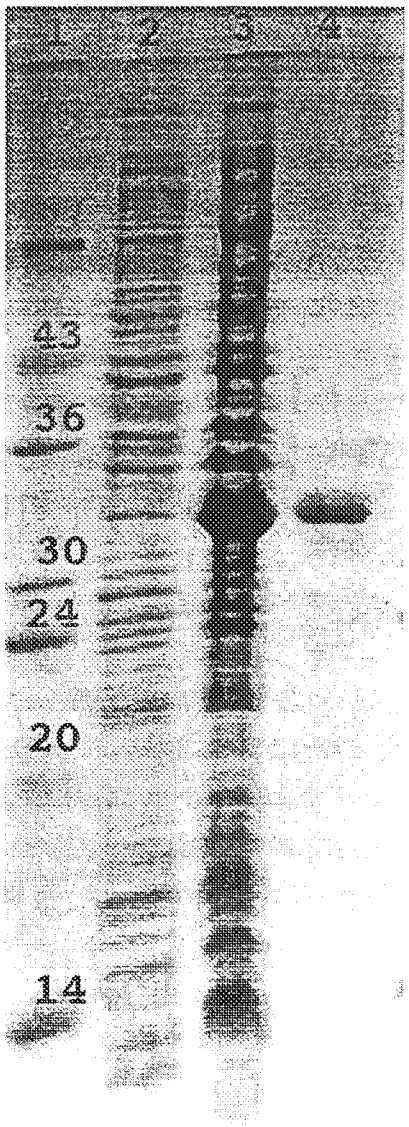

Fig. 1

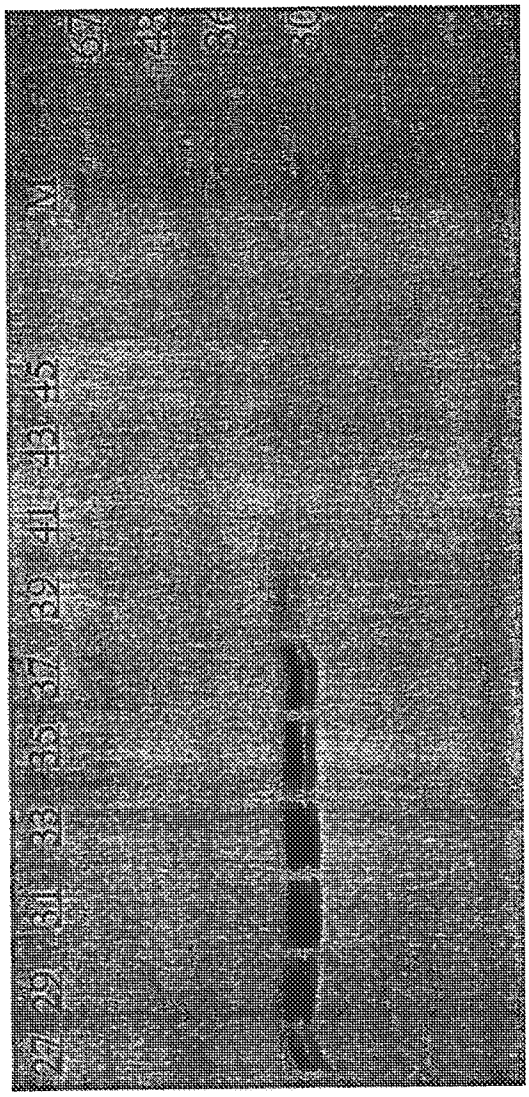

Fig. 2

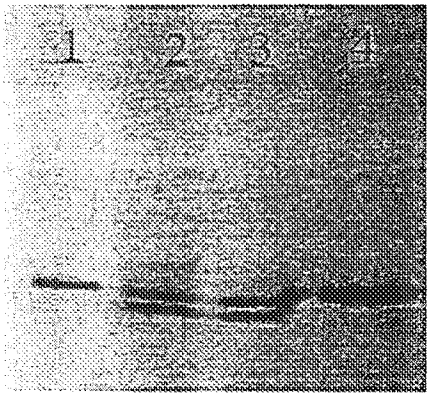

Fig. 3

Fig. 3

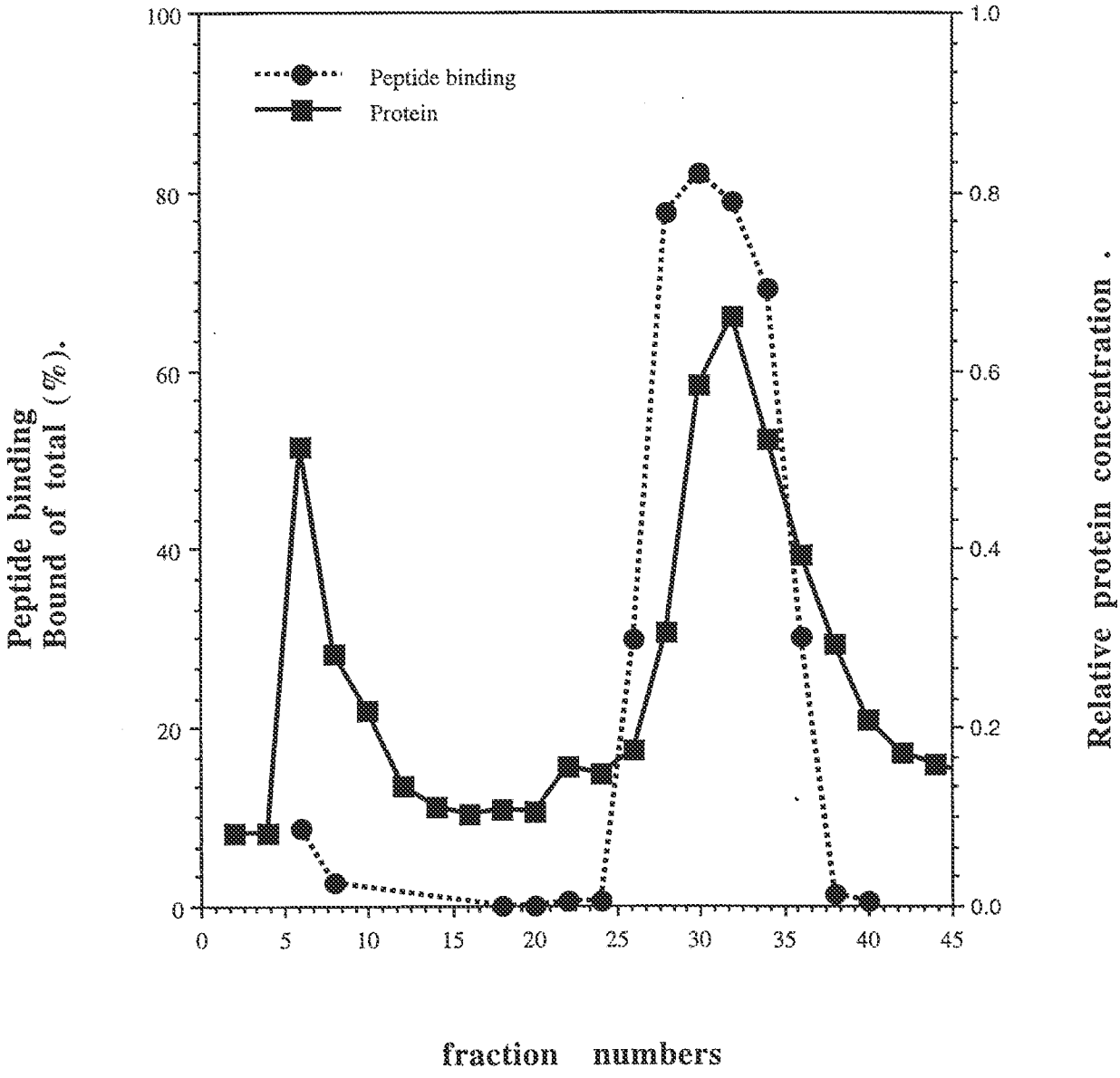
Peptide binding
Protein
Peptide binding
Bound of total (%).
Relative protein concentration .
fraction numbers
0
20
40
60
80
100
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
35
40
45

Fig. 4

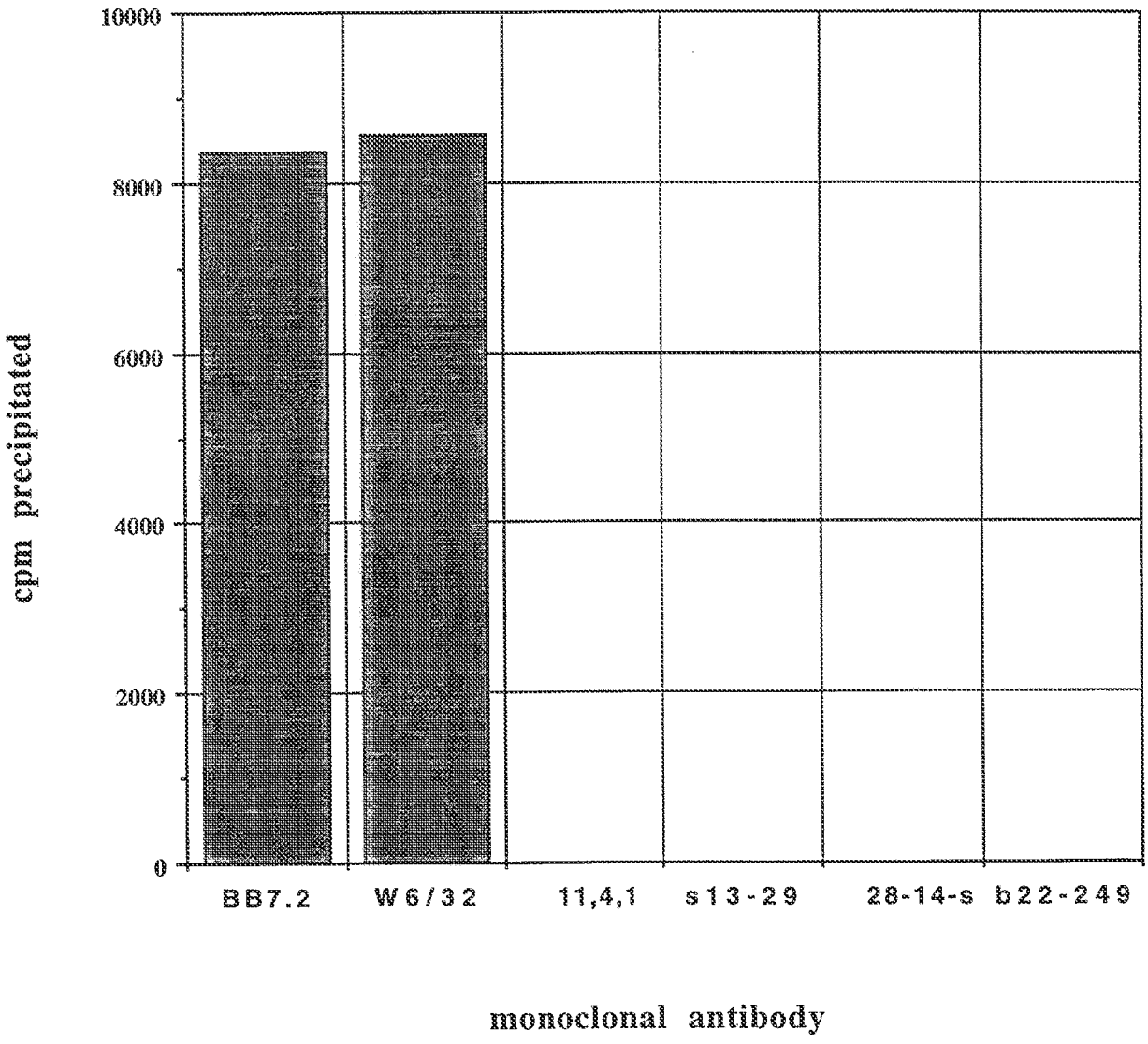
10000
8000
6000
4000
2000
0
cpm precipitated
BB7.2
W6/32
11,4,1
s13-29
28-14-s
b22-249
monoclonal antibody

Fig. 5

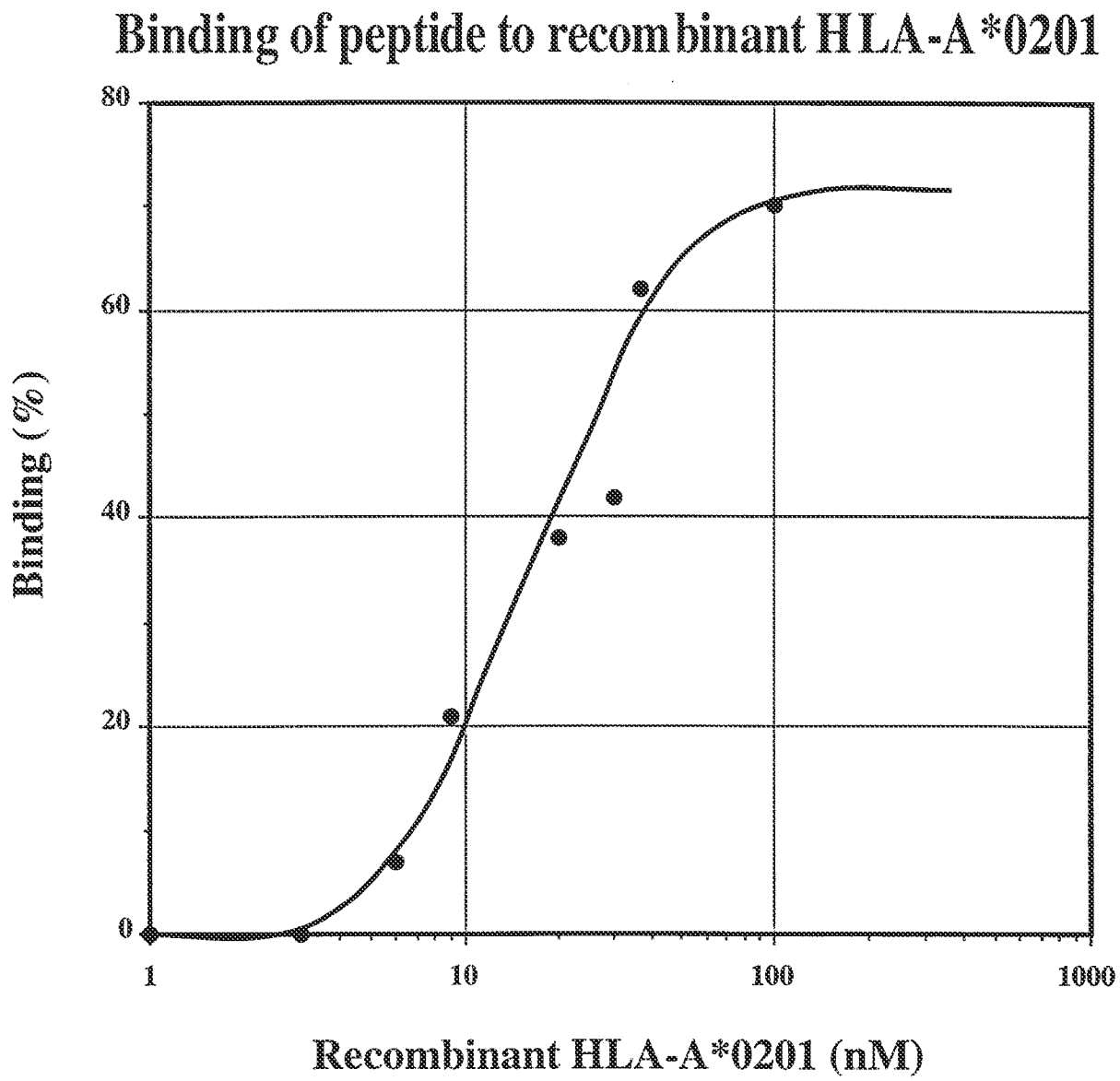
Binding of peptide to recombinant HLA-A*0201
Binding (%)
80
60
40
20
0
1
10
100
1000
Recombinant HLA-A*0201 (nM)

Fig. 6

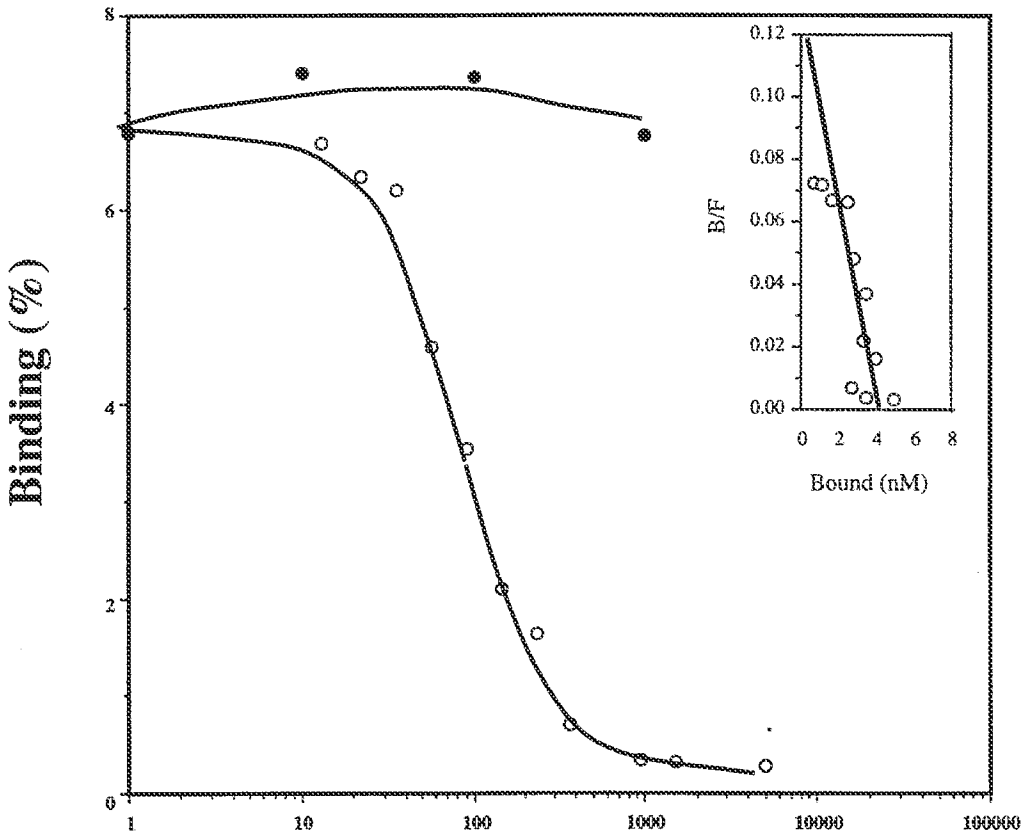
Binding (%)
Peptide (nM)
B/F
Bound (nM)
0
2
4
6
8
1
10
100
1000
10000
100000
0.00
0.02
0.04
0.06
0.08
0.10
0.12

Fig. 7

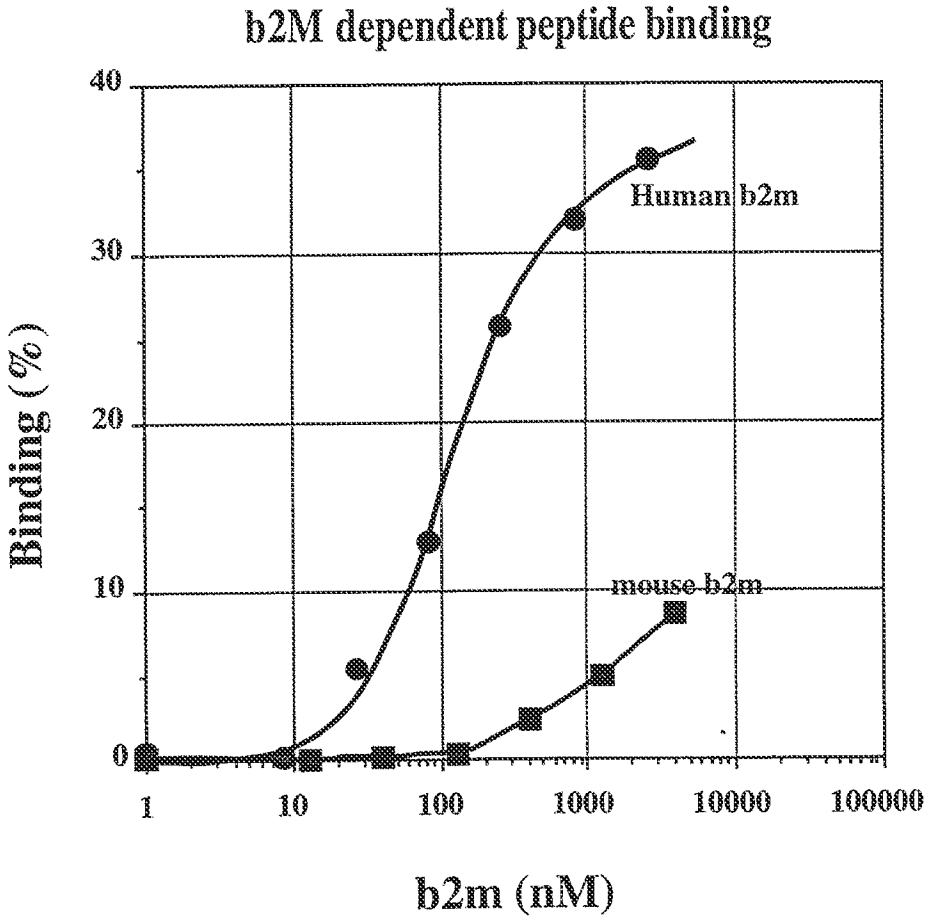
b2M dependent peptide binding
Binding (%)
40
30
20
10
0
1
10
100
1000
10000
100000
b2m (nM)
Human b2m
mouse b2m

Fig. 8

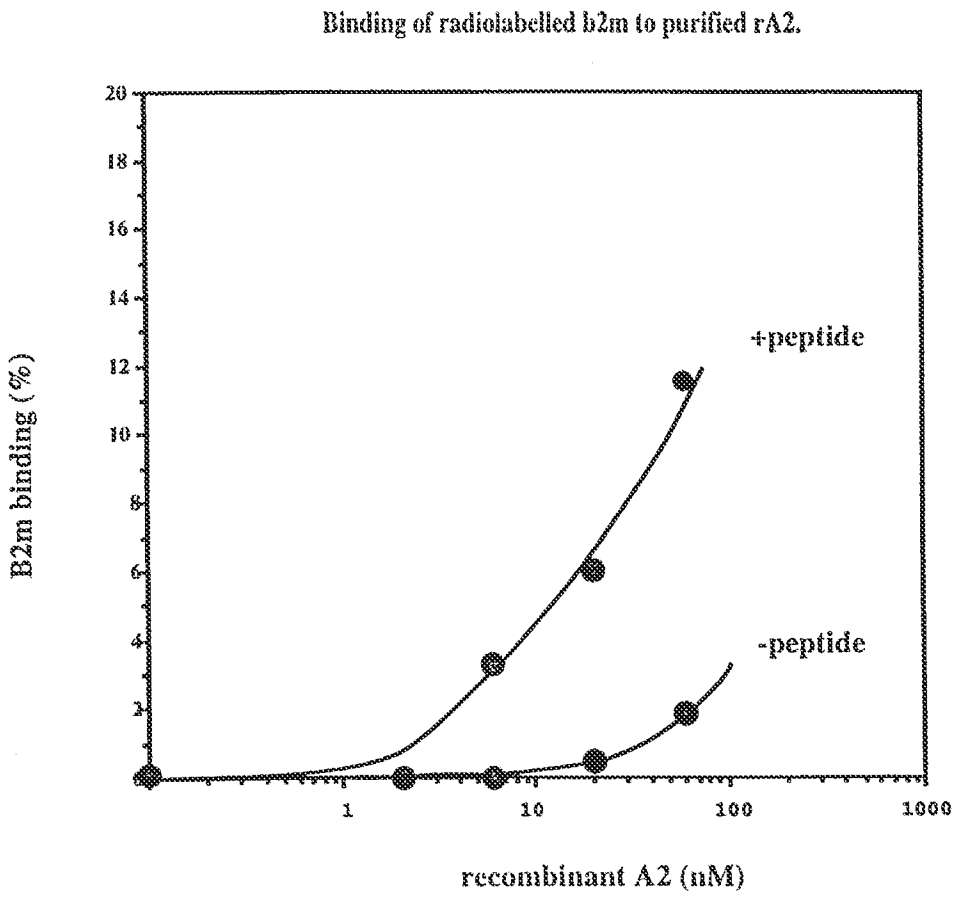
Binding of radiolabelled b2m to purified rA2.
B2m binding (%)
20
18
16
14
12
10
8
6
4
2
0
1
10
100
1000
recombinant A2 (nM)
+peptide
-peptide

Fig. 9

Dissociation of labeled b2m from MHC class I.

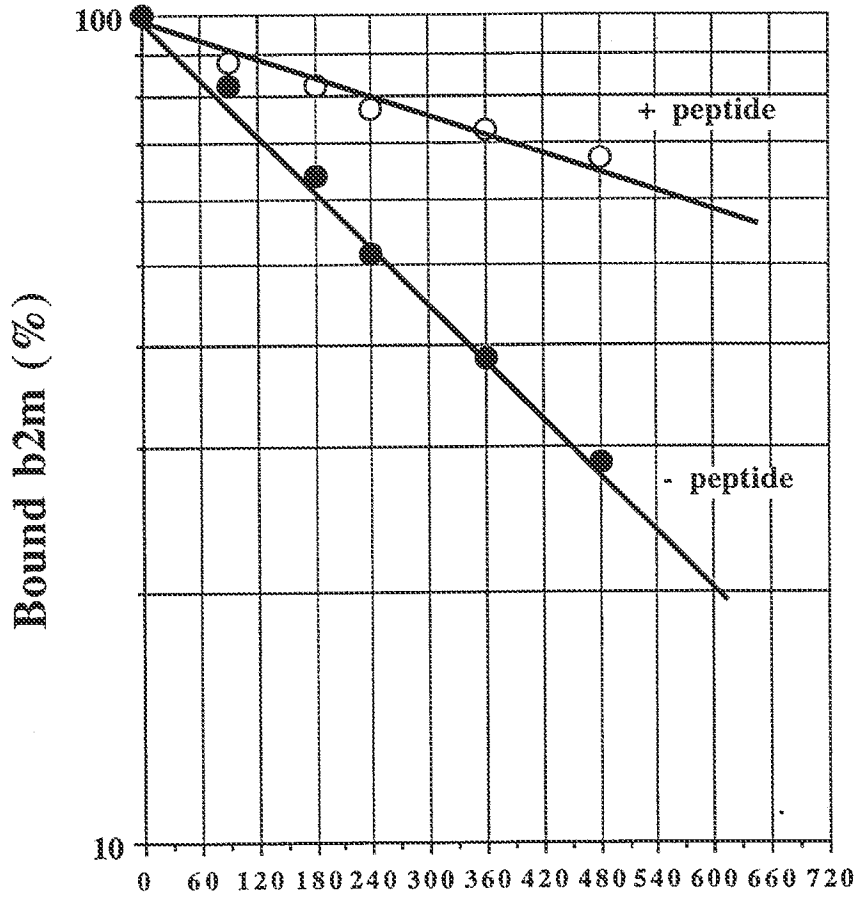

Fig. 10

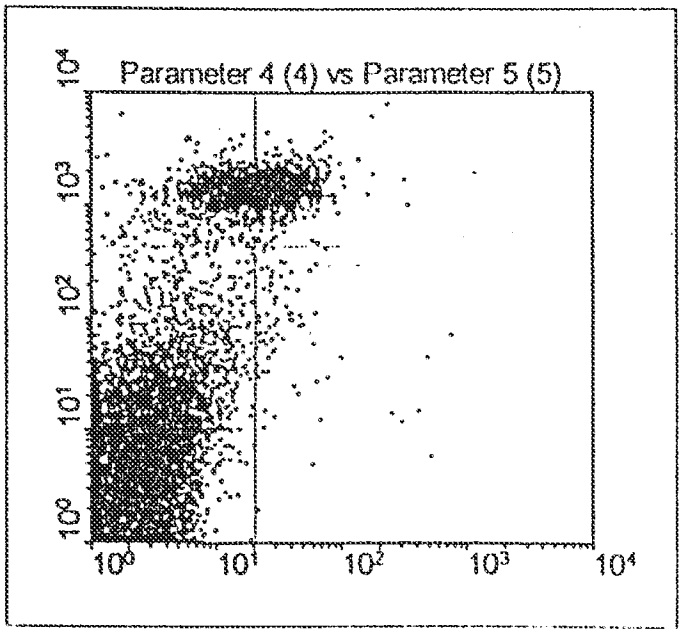

Fig. 11A

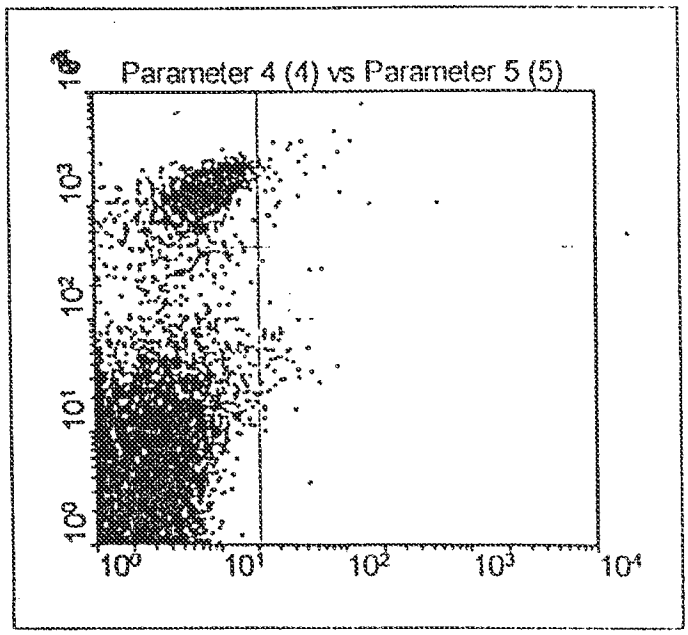

Fig. 11B

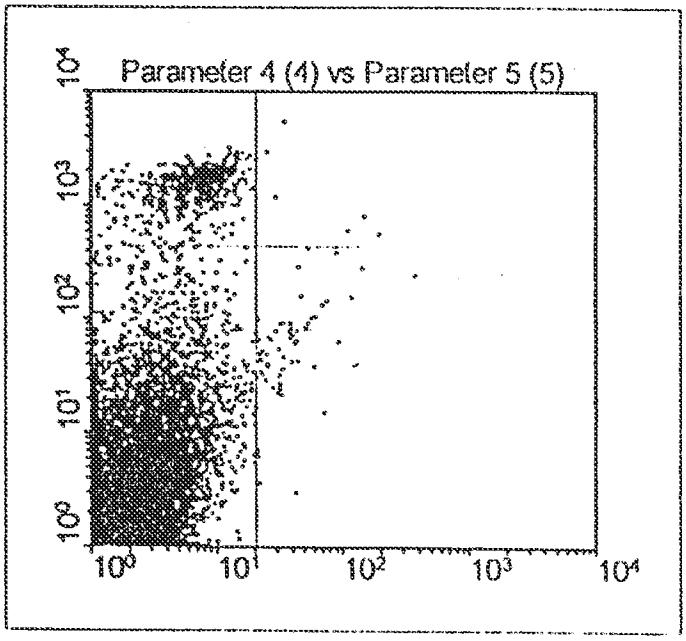

Fig. 11C

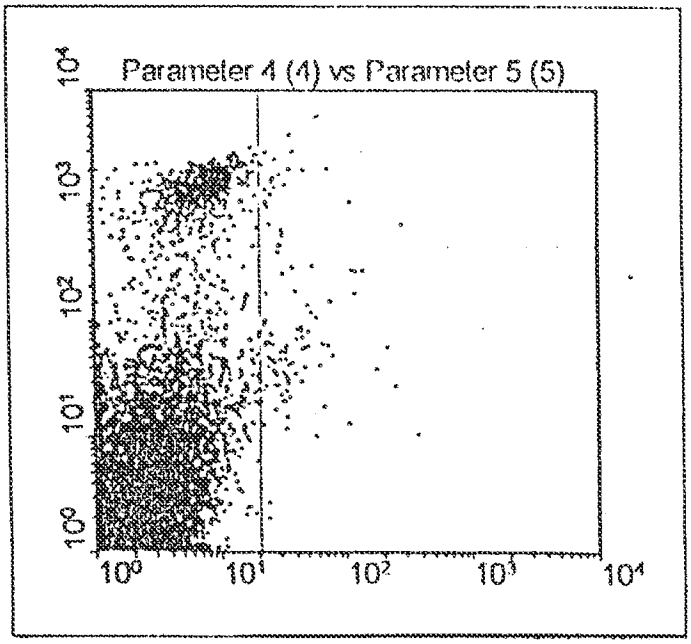

Fig. 11D

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(74) **Agent:** PLOUGMANN, VINGTOFT & PARTNERS A/S; Sankt Annæ Plads 11, P.O. Box 3007, DK–1021 Copenhagen K (DK).

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(54) **Title:** A METHOD OF PRODUCING A FUNCTIONAL IMMUNOGLOBULIN SUPERFAMILY PROTEIN

(57) Abstract

The present invention relates to a process of producing a functional immunoglobulin superfamily protein, which has at least one disulphide bond when functional, the process comprising the steps of providing a bacterial cell, where the gene is expressible, isolating the protein from the cell without reducing it and subjecting the isolated protein to a folding treatment. Preferably, the immunoglobulin superfamily protein is selected from the group consisting of Class I and Class II histocompatibility molecules and beta 2 microglobulin (beta2m). Other embodiments of the invention is a stable peptide free MHC protein obtainable by a process of the invention and a kit comprising a MHC class I heavy chain and a abeta2m allowing the recipient to produce and measure or detect a functional MHC class I protein to which a peptide, which is capabe of binding to said MHC class I protein, can be added leading to the generation of a functional MHC class I protein.

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INTERNATIONAL SEARCH REPORT

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 529 921 A (LANGLADE-DEMOYEN PIERRE ET AL) 25 June 1996 (1996-06-25) column 5, line 21-30 examples 1,5 claim 1	42
A		1-41,49
X	GARBOCZI D N ET AL: "HLA-A2-peptide complexes: refolding and crystallization of molecules expressed in Escherichia coli and complexed with single antigenic peptides." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 APR 15) 89 (8) 3429-33., XP002131059 cited in the application abstract page 3430, left-hand column, line 28-64	1-3, 7-11, 13-41,49

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[X] Further documents are listed in the continuation of box C.

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26 April 2000	17. 05. 2000

Name and mailing address of the ISA	Authorized officer
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Int. Appl. No. PCT/DK 99/00484

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 11702 A (ANERGEN INC) 4 May 1995 (1995-05-04) page 2, line 25 -page 3, line 6 example 2	1-3, 7-11, 13-41,49
X	WO 97 44667 A (INST NAT SANTE RECH MED ;LANGLADE DEMOYEN PIERRE (FR); LONE YU CHU) 27 November 1997 (1997-11-27) page 9, line 25 -page 11, line 30 page 52, line 9 -page 53, line 14 example 1	43-48
X	STEVENS JAMES ET AL: "Efficient generation of major histocompatibility complex class I-peptide complexes using synthetic peptide libraries." JOURNAL OF BIOLOGICAL CHEMISTRY JAN. 30, 1998, vol. 273, no. 5, 30 January 1998 (1998-01-30), pages 2874-2884, XP002136475 ISSN: 0021-9258 abstract page 2875, left-hand column, line 4-11 figures 2,3	50
A	PARKER K C ET AL: "An HLA-A2/beta 2-microglobulin/peptide complex assembled from subunits expressed separately in Escherichia coli." MOLECULAR IMMUNOLOGY, (1992 MAR) 29 (3) 371-8., XP000876800 cited in the application abstract page 372, right-hand column, paragraph 2 -page 373, left-hand column, paragraph 1	1-42,49
A	PLAKSIN D ET AL: "A T cell receptor V alpha domain expressed in bacteria: does it dimerize in solution?." JOURNAL OF EXPERIMENTAL MEDICINE, (1996 OCT 1) 184 (4) 1251-8., XP000876791 abstract page 1252, right-hand column, paragraph 2 -page 1253, left-hand column, paragraph 1	1-42,49

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INTERNATIONAL SEARCH REPORT

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A	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US MOLLOY P E ET AL: "Production of soluble single-chain T-cell receptor fragments in Escherichia coli trxB mutants." retrieved from STN Database accession no. 1998346783 XP002131051 abstract & MOLECULAR IMMUNOLOGY, (1998 FEB) 35 (2) 73-81.,	35-39
A	WO 93 22332 A (UNIV TEXAS ;WARD ELIZABETH SALLY (US); KIM JIN KYOO (US)) 11 November 1993 (1993-11-11) page 6, line 25 -page 7, line 19 page 43, line 1-4	1-42,49
A	WO 94 06813 A (GELIEBTER JAN ;KESARI KRISHNA V (US); UNIV ROCKEFELLER (US)) 31 March 1994 (1994-03-31) page 2, line 1-14 page 13, line 8-27	43-48,50